

# On the role of actomyosin ATPases in regulation of ATP turnover rates during intense exercise

(metabolic control/energy coupling/muscle metabolism/flux control/fiber-type metabolism)

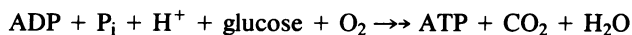
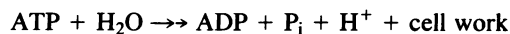
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**ABSTRACT** Actomyosin ATPase is the dominant ATP sink during muscle work. Its catalytic capacities in fast-twitch oxidative glycolytic fibers have long been known to exceed by about 3-fold those of slow-twitch oxidative fibers, but the relative contributions to control of metabolic rates during exercise have never been closely examined. We compared fast-twitch oxidative glycolytic and slow-twitch oxidative fibers that displayed similar mitochondrial abundance (similar activities of mitochondrial marker enzymes). During short-term, but near maximum, aerobic exercise, fast-twitch oxidative glycolytic fibers displayed ATP turnover rates that were 2–4 times higher than for slow-twitch oxidative fibers (despite similar mitochondrial metabolic capacities), implying a large ATPase contribution to control of maximum metabolic rate. Fluxes through the  $\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i$  cycle were extremely well regulated; at the lower limit, the forward flux exceeded the backward flux by only 0.06%, whereas at the upper limit, ATPase rates exceeded ATP synthesis rates by 0.12%. This very high precision of energy coupling could not be easily explained by standard metabolic regulation models.

Current models of metabolic regulation during exercise typically emphasize a crucial role for the energetic status of the cell (expressed as the absolute concentration change in key metabolites such as ADP and ATP, in concentration ratios such as  $\text{ATP}/[\text{ADP}] + [\text{P}_i]$ , or in  $\{\text{PCr}\}$ , defined as the ratio  $[\text{PCr}]/[\text{PCr}] + [\text{Cr}]$  (where PCr is phosphocreatine and Cr is creatine)), with a role for related changes in redox sometimes also assumed to be involved in the regulation of ATP turnover rates (1–4). Connitt *et al.* (4) have argued cogently for a “systems” approach to unraveling metabolic regulatory mechanisms in realistic (i.e., approximating *in vivo*) settings. The two main components of such systems for working muscles are (i) energy-demanding pathways that may involve several interrelated functions (usually ATPases) and (ii) energy-yielding pathways that also may be subdivided into many interlinked (enzymic, diffusive, and convective) functions:



Although the most crucial single regulatory constraint on muscle during any kind of steady-state exercise is the requirement for balancing fluxes through these two pathways, most studies of muscle function regulation during work do not stress (and often seemingly overlook) the boundary conditions imposed by coupling energy demand and energy supply. In consequence most such studies focus upon regulatory roles only of the energy-supply half of the system

under investigation (4). (Exceptions to this are found in studies of fatigue, but in these the systems are usually so far from steady state that normal regulatory mechanisms may no longer be evident.) For these reasons, we decided that it was an opportune time to reevaluate the roles of actomyosin ATPases in the regulation of maximum metabolic rates during muscle exercise. By accounting for some two-thirds of the ATP turnover rates during maximum muscle work (6), the actomyosin ATPases constitute the main energy-demand component of the two-pathway system under analysis. The question arises of whether or not their contributions to control of ATP turnover rates are commensurate with their high ATP demands.

We tried to assess these potential roles for actomyosin ATPases by comparing two kinds of oxidative muscle fibers—slow red and fast red—that have similar oxidative capacities but that have about a 3-fold difference in actomyosin ATPase catalytic potential (7). If a great deal of control were to be vested in this step in ATP turnover then we would expect that at their respective near-maximum work rates, the metabolic rates of these different kinds of muscles should differ greatly (8–10). On the other hand, if maximum metabolic rates were determined by regulation at other sites in the pathways of ATP turnover, then these two kinds of red muscles should display similar maximum metabolic rates, as indeed might be predicted because of similar oxidative capacities (11).

## MATERIALS AND METHODS

**Training and Exercise Protocol.** Adult male Sprague-Dawley rats ( $\approx 300$  g) were trained by running at 30 m/min on a treadmill for 10 min a day, 5 days a week, for 4–8 weeks prior to the experiment. Rats selected for a 2-min high-velocity run were familiarized with the protocol for 7 days before the experiment, always carried out in the morning to avoid any diurnal variation in muscle glycogen (12). The 2-min protocol consisted of running at a treadmill speed of 75 m/min for 20 sec and at 56 m/min for the remaining 1 min 40 sec. At these speeds and the level of training used, the oxidative fibers of hind limb muscles of the rat were considered to be strongly activated to near maximum but sustainable aerobic exercise rates (13); blood flow measurements were available for essentially identical (1 min of steady-state running) conditions (14).

**Anesthesia.** The exercised animals were anesthetized (in 60–90 sec) with an i.p. injection of 1.5 ml containing 0.5 ml of sodium pentobarbital (50 mg/ml) and 1.0 ml of curare (10 mg/ml). Curare, a neuromuscular blocking agent, was found

Abbreviations: FOG, fast-twitch oxidative glycolytic; PCr, phosphocreatine; Cr, creatine;  $\text{V}_{\text{O}_2}$ ,  $\text{O}_2$  consumption.

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to be suitable in preventing muscle twitching after exercise. Nonexercised (control) rats were ether-anesthetized.

**Tissue Preparation.** After anesthesia, the left soleus, plantaris, and the gastrocnemius were excised (in 30, 60, and  $\approx 90$  sec, respectively) and freeze-clamped in liquid nitrogen with precooled aluminum tongs. The same procedure was used for the lower right leg with respective times of 1 min 50 sec, 2 min 15 sec, and 2 min 45 sec. Care was taken not to stretch the muscles during the dissection procedure. Before the homogenization, individual soleus muscles were pooled from the left leg of three rats. An identical procedure was adopted for the three muscles of the right leg. No significant biochemical differences were noted between muscles pooled from either the left or right lower extremities, presumably because in ischemic or poorly perfused muscles that are not doing mechanical work, metabolite concentrations change very slowly (15). Hence, very short sampling times are not as crucial as during sampling of working and well-perfused muscles.

Muscle samples were powdered under liquid nitrogen and extracted as described (16). Glycolytic intermediates were measured using coupled enzymatic procedures (16). The concentrations of ATP, ADP, AMP, IMP, PCr, and Cr were analytically determined by HPLC (16);  $P_i$  was determined by a colorimetric method (17). This method was subsequently validated with  $^{31}\text{P}$  NMR on neutralized muscle extracts from resting and exercising fish (W.S.P. and G.P.D., unpublished data). Intracellular pH of muscle was estimated by using a homogenate technique (18).

**Enzyme Measurements.** To estimate relative oxidative metabolic capacities of fast twitch oxidative glycolytic (FOG or fast red) fiber types vs. slow oxidative (or slow red) fiber types in this species, four mitochondrial marker enzymes were measured (19, 20): carnitine palmityltransferase, hydroxyacyl-CoA dehydrogenase, citrate synthase, and glutamate dehydrogenase. Soleus ( $\approx 89\%$  slow oxidative fibers) was used as a source of slow red fibers; red gastrocnemius and red vastus were used as two alternative sources of fast red fibers (21). The red gastrocnemius was separated from the rest of this muscle bundle for the enzyme determinations, but for technical reasons not for metabolite measurements.

## RESULTS

**Enzyme Potentials.** All four enzyme activities in red soleus and red gastrocnemius were within 20% of each other (Table 1). Although this was our main comparison for estimating the relative oxidative capacities of FOG and slow-twitch oxidative fibers, it is also noteworthy that red vastus (composed mainly of FOG fibers) displayed similar enzyme activities per g of tissue. Since the enzymes were assayed under identical conditions (the values presumably reflecting relative mitochondrial abundance), we assumed that in this species the oxidative catalytic capacities of fast red and slow red muscles

(including FOG fibers of plantaris that could not be measured directly but was used in the metabolite studies below) are similar (Table 1), in agreement with mitochondrial volume density measurements made by several other studies (see ref. 22). Actomyosin ATPase catalytic potentials, however, differ by a factor of 3 or even more. For purposes of this analysis, we have assumed that the catalytic activity of actomyosin ATPase in fast red muscle is 350  $\mu\text{mol}$  of ATP per g of muscle per min at normal body temperatures compared to a value of 117  $\mu\text{mol}$  of ATP split per g of muscle per min for slow red muscles such as the soleus (7, 23, 24). Numerous studies have repeatedly demonstrated close to a 3-fold difference between fast red and slow red muscles in mammals and birds, including man (for example, see refs. 23 and 24).

**Metabolite Profiles.** Metabolite concentrations at rest and at 2 min of running (Tables 2 and 3) were determined in soleus and compared to two muscles with different amounts of FOG fibers: the red gastrocnemius (30% FOG) and the plantaris (50% FOG). Enzyme activities of FOG fibers in plantaris could not be determined directly and were assumed to be similar to FOG fibers in red vastus and red gastrocnemius. For soleus, the change in glycogen was equal to 10  $\mu\text{mol}$  per g of tissue per 2 min, whereas for the gastrocnemius and the plantaris the change was equal to almost twice this value. The gastrocnemius accumulated lactate to concentrations of about 13  $\mu\text{mol/g}$  of tissue, whereas the soleus accumulated only about 7  $\mu\text{mol/g}$  of tissue in the 2 min of running. The degree of perturbation of adenylate, PCr, and Cr concentrations also was compared for all three muscles; intramuscular pH values, however, could only be obtained for gastrocnemius and plantaris (because of the small size of the soleus, intramuscular pH could not be measured without sacrificing other determinations).

**Metabolic Rate Calculations of Various Muscles During Maximum Exercise.** During the first 5 min or so of high work rates in rat skeletal muscles, essentially all of the required energy is derived from glycogen catabolism (25, 26). Fat catabolism is minimal at this time (which is in fact a common metabolic organization in most vertebrate muscles) and when it does kick in, its contributions are similar in muscles of different fiber composition, at least over short periods (26). Thus we estimated *in vivo* metabolic rates (expressed in terms of ATP turned-over per g of muscle per min) by calculating the amount of glycogen used, the amount glycolyzed to lactate, and the amount accumulated as intermediates. (PCr contributions were ignored because we assumed they were important only in very first phases of exercise; including them would not change our overall values significantly.) In the limiting or boundary situation, we assumed that all the lactate formed during 2 min of running work was retained in the muscles (at sites of formation with quantitatively insignificant efflux into the blood). A second assumption was that some fraction of the glycogen being depleted was not fully oxidized but was being retained in muscle tissue in the form of pathway intermediates. The most important of these were glucose 6-phosphate and the rest of the glycolytic intermediates (Table 3) and alanine (27). From our measurements (Table 3), we estimated that in the gastrocnemius just under 2  $\mu\text{mol/g}$  of glucosyl carbon was tied up as pathway intermediates, and from previous studies (27) we assumed that about 2  $\mu\text{mol/g}$  was retained as alanine; thus, for the 2-min run, we calculated that 16  $\mu\text{mol/g}$  of glucosyl glycogen was metabolized by the gastrocnemius, or 8  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ .

For the plantaris (Table 3), which accumulated lower levels of glycolytic intermediates and presumably similarly lower amounts of alanine, we assumed a total of 1  $\mu\text{mol}$  of glycogen carbon per g of muscle was retained; thus its metabolic rate in terms of glucosyl glycogen units was estimated at 9  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ . For the soleus, the amount of glycogen tied up in intermediates also was less than in the gastrocnemius

Table 1. Maximum catalytic activities of mitochondrial marker enzymes in rat muscles

Enzyme	Activity, $\mu\text{mol}$ of substrate converted per g of muscle per min		
	Soleus	Red gastrocnemius	Red vastus
CS	25.95 $\pm$ 2.85	31.79 $\pm$ 2.60	32.39 $\pm$ 3.02
CPT	3.03 $\pm$ 0.63	2.60 $\pm$ 0.47	2.96 $\pm$ 0.30
HOAD	27.86 $\pm$ 3.99	27.52 $\pm$ 3.43	27.08 $\pm$ 3.47
GDH	4.58 $\pm$ 0.67	4.46 $\pm$ 0.59	4.70 $\pm$ 0.93

Data were collected at 37°C and are expressed as mean  $\pm$  SEM ( $n = 6$ ). CS, citrate synthase; CPT, carnitine palmityltransferase; HOAD, hydroxyacyl-CoA dehydrogenase; GDH, glutamate dehydrogenase.

Table 2. Changes in fuel, nucleotide concentration, and pH in rat skeletal muscles

Metabolite	Metabolite concentration, $\mu\text{mol/g}$ (wet weight) of muscle					
	Gastrocnemius		Plantaris		Soleus	
	Control (5)	Exercise (5)	Control (6)	Exercise (6)	Control (5)	Exercise (6)
PCr pool						
PCr	17.71 $\pm$ 0.42	9.83 $\pm$ 0.65 (S)	15.50 $\pm$ 1.49	8.39 $\pm$ 0.99 (S)	9.29 $\pm$ 0.93	6.67 $\pm$ 0.75 (S)
Cr	28.96 $\pm$ 0.17	39.02 $\pm$ 0.81 (S)	25.74 $\pm$ 1.03	35.24 $\pm$ 1.50 (S)	22.11 $\pm$ 4.36	25.94 $\pm$ 1.65 (NS)
Total	46.67 $\pm$ 0.34	48.85 $\pm$ 0.55 (NS)	41.24 $\pm$ 2.13	43.63 $\pm$ 1.00 (NS)	31.40 $\pm$ 1.85	32.61 $\pm$ 1.58 (NS)
Glycogen pool						
Glycogen	38.56 $\pm$ 2.19	18.39 $\pm$ 1.50 (S)	32.90 $\pm$ 1.19	13.99 $\pm$ 1.21 (S)	24.70 $\pm$ 1.47	14.48 $\pm$ 0.85 (S)
Lactate	2.58 $\pm$ 0.57	13.26 $\pm$ 1.60 (S)	3.22 $\pm$ 0.28	11.40 $\pm$ 1.94 (S)	3.84 $\pm$ 0.80	7.26 $\pm$ 0.51 (S)
Nucleotide and phosphate pool						
ATP	6.57 $\pm$ 0.08	6.02 $\pm$ 0.402 (NS)	5.73 $\pm$ 0.15	5.28 $\pm$ 0.23 (NS)	3.77 $\pm$ 0.167	3.94 $\pm$ 0.157 (NS)
ADP	0.915 $\pm$ 0.036	0.864 $\pm$ 0.026 (NS)	0.871 $\pm$ 0.056	0.953 $\pm$ 0.079 (NS)	0.815 $\pm$ 0.043	0.847 $\pm$ 0.029 (NS)
AMP	0.031 $\pm$ 0.005	0.029 $\pm$ 0.004 (NS)	0.045 $\pm$ 0.004	0.052 $\pm$ 0.008 (NS)	0.069 $\pm$ 0.010	0.076 $\pm$ 0.018 (NS)
IMP	0.046 $\pm$ 0.007	0.654 $\pm$ 0.196 (S)	0.211 $\pm$ 0.058	0.686 $\pm$ 0.186 (NS)	0.164 $\pm$ 0.109	0.115 $\pm$ 0.024 (NS)
Total	7.56 $\pm$ 0.05	7.57 $\pm$ 0.34 (NS)	6.86 $\pm$ 0.25	6.97 $\pm$ 0.06 (NS)	4.82 $\pm$ 0.186	4.98 $\pm$ 0.167 (NS)
P <sub>i</sub>	14.10 $\pm$ 0.28	19.47 $\pm$ 0.92 (S)	14.39 $\pm$ 1.37	17.91 $\pm$ 1.39 (S)	12.51 $\pm$ 1.06	12.52 $\pm$ 0.47 (NS)
NH <sub>4</sub> <sup>+</sup>	0.231 $\pm$ 0.057	1.00 $\pm$ 0.253 (S)	0.700 $\pm$ 0.250	0.744 $\pm$ 0.151 (NS)	0.714 $\pm$ 0.070	1.474 $\pm$ 0.385 (S)
pH	6.92 $\pm$ 0.04	6.63 $\pm$ 0.04 (S)	6.92 $\pm$ 0.07	6.62 $\pm$ 0.03 (S)	—	—

Metabolite concentrations are given as mean  $\pm$  SEM with the number of rats sampled in parentheses. Statistical significance (*P*) between each exercise (2-min run) and control group was evaluated using the two-tailed Student's *t* test. S, significant (*P* < 0.05); NS, not significant.

(Table 3); in total 1  $\mu\text{mol}$  of glycolytic intermediates and 1  $\mu\text{mol}$  of alanine per g of muscle was considered a reasonable estimate. Thus the soleus glucosyl glycogen metabolic rate was estimated at 4.5  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ . If no lactate was effluxed, the metabolic rates of the gastrocnemius, plantaris, and soleus would have been 211, 265, and 132  $\mu\text{mol}$  of ATP turned over per g of muscle per min (Table 4).

The above calculations did not take into account lactate kinetics or percent FOG fiber composition of the gastrocnemius and plantaris ( $\approx 50$  and 30%, respectively). To correct for lactate efflux, we assumed 300  $\mu\text{mol}$  of lactate per 300 g (body weight) per min for rats under heavy-exercise conditions (28). To factor in the effect of FOG fibers, we assumed

[along with Armstrong and Laughlin (13, 14)] that most aerobic metabolic processes in extreme exercise are localized to FOG fibers (which constitute some 25% of the limb musculature of rats). We considered this to be reasonable since the fraction of slow red fibers in rat limb muscles is low (10% or less) and under comparable working conditions the perfusion of FOG fibers may account for most of the limb muscle blood flow and could exceed by 6–8 times the perfusion of fast-twitch glycolytic fibers (14). Terjung and his coworkers (29) similarly assumed that fast-twitch glycolytic fibers under these conditions do not participate in oxidatively supported work; this allowed them to estimate FOG fiber specific O<sub>2</sub> consumption rates during electrical stimulation.

Table 3. Glycolytic intermediates in rat skeletal muscles

Metabolite	Metabolite concentration, $\mu\text{mol/g}$ (wet weight) of muscle					
	Gastrocnemius		Plantaris		Soleus	
	Control (5)	Exercise (5)	Control (6)	Exercise (6)	Control (5)	Exercise (6)
Glucose	0.467 $\pm$ 0.052	1.08 $\pm$ 0.05 (S)	0.460 $\pm$ 0.022	0.688 $\pm$ 0.147 (S)	0.971 $\pm$ 0.089	1.46 $\pm$ 0.06 (S)
Glucose 1-phosphate	0.044 $\pm$ 0.009	0.079 $\pm$ 0.013 (S)	0.065 $\pm$ 0.008	0.079 $\pm$ 0.007 (NS)	0.014 $\pm$ 0.005	0.018 $\pm$ 0.004 (NS)
Glucose 6-phosphate	0.784 $\pm$ 0.171	1.87 $\pm$ 0.25 (S)	0.525 $\pm$ 0.038	0.678 $\pm$ 0.047 (S)	0.431 $\pm$ 0.071	0.695 $\pm$ 0.045 (S)
Fructose 6-phosphate	0.137 $\pm$ 0.073	0.367 $\pm$ 0.062 (S)	0.211 $\pm$ 0.019	0.300 $\pm$ 0.024 (S)	0.080 $\pm$ 0.017	0.158 $\pm$ 0.016 (S)
Fructose 1,6-bisphosphate	0.348 $\pm$ 0.031	0.111 $\pm$ 0.021 (S)	0.296 $\pm$ 0.033	0.186 $\pm$ 0.021 (S)	0.156 $\pm$ 0.024	0.132 $\pm$ 0.012 (NS)
Dihydroxyacetone phosphate	0.062 $\pm$ 0.005	0.048 $\pm$ 0.006 (S)	0.074 $\pm$ 0.003	0.058 $\pm$ 0.004 (S)	0.056 $\pm$ 0.003	0.043 $\pm$ 0.006 (NS)
Glyceraldehyde 3-phosphate	0.028 $\pm$ 0.005	0.017 $\pm$ 0.002 (NS)	0.037 $\pm$ 0.006	0.023 $\pm$ 0.004 (NS)	0.036 $\pm$ 0.006	0.031 $\pm$ 0.001 (NS)
1,3-Bisphosphoglycerate	0.030 $\pm$ 0.006	0.024 $\pm$ 0.001 (NS)	0.029 $\pm$ 0.006	0.023 $\pm$ 0.003 (NS)	0.055 $\pm$ 0.007	0.026 $\pm$ 0.005 (S)
3-Phosphoglycerate	0.182 $\pm$ 0.014	0.130 $\pm$ 0.011 (S)	0.203 $\pm$ 0.011	0.166 $\pm$ 0.006 (S)	0.165 $\pm$ 0.010	0.164 $\pm$ 0.007 (NS)
2-Phosphoglycerate	0.013 $\pm$ 0.001	0.011 $\pm$ 0.004 (NS)	0.028 $\pm$ 0.004	0.013 $\pm$ 0.004 (S)	0.040 $\pm$ 0.006	0.028 $\pm$ 0.009 (NS)
Phosphoenolpyruvate	0.048 $\pm$ 0.004	0.029 $\pm$ 0.007 (S)	0.063 $\pm$ 0.002	0.049 $\pm$ 0.003 (S)	0.065 $\pm$ 0.016	0.060 $\pm$ 0.004 (NS)
Pyruvate	0.089 $\pm$ 0.009	0.091 $\pm$ 0.009 (NS)	0.133 $\pm$ 0.014	0.112 $\pm$ 0.009 (NS)	0.128 $\pm$ 0.012	0.117 $\pm$ 0.009 (NS)
Lactate	2.58 $\pm$ 0.57	13.26 $\pm$ 1.60 (S)	3.22 $\pm$ 0.28	11.40 $\pm$ 1.90 (S)	3.84 $\pm$ 0.80	7.26 $\pm$ 0.51 (S)

Metabolite concentrations are given as mean  $\pm$  SEM with the number of rats sampled in parentheses. Statistical significance (*P*) between each exercise (2-min run) and control group was evaluated by using the two-tailed Student's *t* test. S, significant (*P* < 0.05); NS, not significant.

Table 4. Calculated ATP turnover rates assuming no lactate efflux

Muscle	Calculated ATP turnover	
	From glycolysis	From oxidative metabolism
Soleus	3.0	129
Plantaris	6.0	259
Gastrocnemius	7.5	204

ATP turnover is expressed as  $\mu\text{mol}$  of ATP per g per min, assuming all lactate formed in the 2-min run was retained in muscle.

Using this framework, we estimated maximum lactate efflux rates during the 2-min run of 12  $\mu\text{mol}$  of lactate per g of FOG fibers per min [muscle weight of a 300-g rat was taken to be 100 g, 25% of which was taken to be FOG fibers (14, 21)]. For the gastrocnemius, this meant that glycogen carbon flow to lactate was 5  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$  (retained in muscle) plus 4  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$  effluxed, requiring a total of 4.5  $\mu\text{mol}$  of glucosyl units $\cdot\text{g}^{-1}\cdot\text{min}^{-1}$  and yielding 13.5  $\mu\text{mol}$  of ATP in the process. Oxidation of the remaining 3.5  $\mu\text{mol}$  of glucosyl glycogen by 0.3 g of FOG fibers yielded an oxidative metabolic rate of 432  $\mu\text{mol}$  of ATP per g of FOG fibers per min ( $3.5 \times 37/0.3$ ). This more realistic set of values implied a glycolytic contribution of 13.5/432 or 3.1% of the oxidative rate (Table 5). Similar calculations for the plantaris (50% FOG fibers) indicated an oxidative metabolic rate of 296  $\mu\text{mol}$  of ATP per g of FOG fibers per min and a glycolytic contribution that was about 5% as large or 15  $\mu\text{mol}$  of ATP per g per min. These values of oxidative metabolic rates were, respectively, 2.3 and 3.3 times larger than the metabolic rate calculated for the soleus (Table 5).

If our estimates of lactate efflux were too high [which is probable (31)], then even a greater proportion of the glycogen utilized was presumably oxidized; in this case, our calculations would have underestimated the ATP turnover rates in fast red muscles. If our assessments of lactate efflux were too low (as in soleus where we assumed the process to be so slow that it could be ignored over a 2-min exercise bout), then our calculations would have overestimated the ATP turnover rate. This means that the metabolic rate differences between the soleus on the one hand and the gastrocnemius and plantaris on the other were minimized by these calculations, not vice versa (Table 5).

## DISCUSSION

A number of unexpected and potentially interesting implications arise from this analysis.

**Comparison of ATP Turnover Rates.** The values of oxidative metabolic rate calculated for FOG fibers in this study seem unexpectedly high and the first matter to consider is how these rates correlate with whole-organism maximum  $\text{O}_2$

consumption ( $\dot{V}\text{O}_2$ ) measurements. Although the literature values for maximum  $\dot{V}\text{O}_2$  rates of exercising rats vary somewhat, 80 ml of  $\text{O}_2$  per kg per min is a fairly widely accepted estimate (see ref. 32). The hind limb muscles of a 300-g rat weigh about 30 g in total, so we have assumed about 50 g of locomotory musculature. Hence a  $\dot{V}\text{O}_2$  value of 80 ml of  $\text{O}_2$  per kg per min is equivalent to 20  $\mu\text{mol}$  of  $\text{O}_2$  per g of locomotory muscle per min or 120  $\mu\text{mol}$  of ATP per g per min. Since about 25% of this musculature is composed of FOG fibers [and most of the rest is composed of fast-twitch glycolytic fibers thought to play a minimal role in sustained metabolism and work (13, 14)], it is possible for fast red muscles to achieve rates of oxidative metabolism equal to 480  $\mu\text{mol}$  of ATP per g of FOG fibers per min; these values approach those of hummingbird flight muscles during hovering (30) and may require a significant fraction of available actomyosin ATPase activity (Table 5).

**Mitochondrial Oxidative Capacities and ATP Turnover.** Perhaps the most surprising result of these studies is the large difference in *in vivo* ATP turnover rates sustained by fast red and slow red muscles; it is minimally  $\approx 2$ -fold (Table 4), but realistically is closer to 3- to 4-fold (Table 5). At first glance, this is unexpected because the oxidative capacities of slow red and fast red muscles in the rat are almost indistinguishable from each other. If mitochondrial volume densities are similar (22, 33) as are the catalytic activities of mitochondrial marker enzymes (Table 1), then it is not reasonable to expect maximum  $\text{O}_2$ -linked ATP turnover rates also to be similar? Although this has been a debated issue in recent years, the general consensus from between-species comparisons is that maximum  $\dot{V}\text{O}_2$  correlates well with mitochondrial abundance (11, 22, 33). Our results however clearly show that the mitochondrial-based ATP turnover rates of FOG fibers exceed by far those of slow oxidative muscles, such as the soleus (Table 5). In the language of control theory (10), this is because the energy-demand pathways of the system (either actomyosin ATPases alone or in combination with  $\text{Ca}^{2+}$  ATPases and other ATP-utilizing reactions) contribute in a major way to flux regulation particularly as the system approaches maximum velocities.

**Regulatory Insights.** In considering the overall regulation of ATP turnover during the 2-min run, the question arises of how closely matched are the fluxes through muscle ATPases and muscle ATP synthases. How tightly coupled are the energy-demand and energy-supply pathways of the systems under analysis? For the gastrocnemius and plantaris, fairly rich in fast fibers, a quantitative answer to this question arises from IMP concentrations. Because of the stoichiometry between ATP depletion and IMP accumulation and because of high AMP deaminase activities in fast twitch fibers (34), IMP accumulation through the 2-min run becomes a fairly accurate index of the degree to which ATPase fluxes exceed ATP synthase fluxes. Since the accumulation observed is in fact relatively modest (Table 2), energy-demand and energy-supply pathways in these muscles remained well coupled throughout the exercise protocol: while fluxing nearly 500  $\mu\text{mol}$  of ATP per g per min in both ATPase and ATP synthase directions (Table 5), these muscles accumulated only  $\approx 0.15$   $\mu\text{mol}$  of IMP per g per min (Table 2). At this metabolic rate, for each 1666 turns of the  $\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i$  cycle, in only one does substrate escape to IMP—a surprising regulatory precision. Even if we assume the lower metabolic rate of about 250  $\mu\text{mol}$  of ATP per g min (Table 4), the flux imbalance remains low: in this case, for each 833 turns of the  $\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i$  cycle, one turn allows substrate escape to IMP. This means that the error signal—the degree to which ATPase flux exceeds ATP synthase flux—is only 0.06–0.12% whereas the overall metabolic rates, especially of FOG fibers, may be two orders of magnitude higher than at rest.

Table 5. Comparative oxidative metabolic rates

Muscle type	ATP turnover rate	Actomyosin ATPase	Flow rate
Slow oxidative fibers (soleus)	129	117	233
FOG (plantaris)	296	350	215
FOG (gastrocnemius)	432	350	403
FOG (hummingbird flight muscle)*	490	350	

ATP turnover is expressed as  $\mu\text{mol}$  of ATP per g per min; see text for calculations. Actomyosin ATPase is from Marston and Taylor (7), assuming a  $Q_{10}$  of 2.5. Flow rate is from Armstrong and Laughlin (14) and is expressed as ml per 100 g per min.

\*From Suarez *et al.* (30) based on  $\text{O}_2$  consumption rates during hovering flight.

What kind of regulatory mechanisms could account for such precision?

Several current models of metabolic regulation (1–4) would predict that changes in concentrations of adenylates, PCr, Cr,  $P_i$ ,  $H^+$ , and/or NADH/NAD $^+$  are able to mediate observed changes in ATP cycling rates. These models typically incorporate both kinetic and thermodynamic components. Although it is often either unstated or overlooked, only the former can directly regulate reaction rates (by enzyme–substrate interactions controlled by effectors or modulators) while change in thermodynamic components (such as redox or phosphorylation potentials) may correlate with, but cannot directly cause, change in reaction rates. In neither event, however, are our data and analyses consistent with these traditional models. The first problem is that our data on such parameters cannot readily account for the different metabolic rates observed in the three muscles examined. Instead in comparing the three muscles, it is the relative similarity, not the magnitude of difference, in these parameters that is most striking about the data. On face value, these data are inconsistent with the “driving functions” proposed for these parameters by current regulation models.

A second problem arises from a key requirement of current models (cast in Michaelis–Menten form) that changes in metabolic rate are directly related to the proposed driving functions. To be sure, good correlations between some parameters (such as the free concentration of ADP) and metabolic activation are obtained in some studies (1–4), but these are not always 1:1 relationships; usually a small change in free ADP concentration correlates with a substantially larger change in  $\dot{V}O_2$  or in work output (1). In an independent study of electrically stimulated muscle, we found that the correlation between free ADP concentration and ATP turnover rate depended on  $O_2$  availability, and again, the percent change in  $\dot{V}O_2$  exceeded the relative change in free ADP concentration. Similar relative stability of putative regulators (especially of the adenylates) during large changes in metabolic and work rates in fact are not uncommon (1, 3).

For these reasons, we conclude that available data repeatedly point out two crucial problems: (i) not enough change in signal to account (by the Michaelis–Menten models currently used) for many of the extreme metabolic rates observed in working muscle and (ii) not enough sensitivity (or gain) to account for the precise energy coupling observed. We consider the latter particularly difficult for current models of regulation, because nowhere in our data or in data published by others (1, 4) do we see convincing evidence of the enormous sensitivity or gain required to respond to 0.06–0.12% change in error signal in forward and back ATP flux rates. That is the main reason we consider that the situation may be more analogous to that found in insect flight muscles: during flight these muscles can sustain two to three orders of magnitude increase in flux through the glycolytic pathway with modest (sometimes immeasurable) change in the concentration of pathway intermediates (5). Such regulatory precision is not dependent upon mass-action-regulated enzyme mechanisms but instead is achieved by finely tuned allosteric regulation or covalent modification of key enzymes in the pathway. The major advantage of such control is that it allows a very large magnitude change in the flux rate with minimal or no change in substrate and product concentrations. Similar mechanisms may be operative in the regulation of oxidative metabolic rates in working mammalian muscles, but because of fiber type and metabolic complexity of the system these may have been overlooked. If that should be the case, then the observed changes during longer-term work in so called regulatory parameters of oxidative metabolism

[which undoubtedly are observed (4)] may merely be reflecting changes in ATP cycling rates rather than causing them. Be that as it may, the question of how ATP synthesis rates so closely balance ATPase rates, although of immense interest, remains unanswered.

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